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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/086,542	02/28/2002	Geoffrey M. Wahl	SALK1790-6 (088802-3457)	2411

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EXAMINER	
BERTOGLIO, VALERIE E	

ART UNIT	PAPER NUMBER
1632	7

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/086,542	WAHL ET AL.	
	Examiner	Art Unit	
	Valarie Bertoglio	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-19* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-19* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *Detailed Action* .

DETAILED ACTION

Claims 1-19 are pending and currently under consideration.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-19 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The transgenic non-human animal claimed is not disclosed as to any particular characteristics and there is no example descriptive of how to make the invention defined by claims 1-19. The claimed inventions encompass transgenic animals for three strategies of transgene manipulation through FLP-mediated homologous recombination at FRT sites. In the first strategy, an FRT site within the genome of a mammal is a site for recombination and site-specific insertion of a transgene (Claim 1). In a second strategy, a gene interrupted by an FRT flanked disruption is placed within the genome and FLP-induced homologous recombination between FRT sites removes the disrupting DNA, providing a functional gene (claim 12). A third strategy a FRT site is placed within a gene in the genome such that the gene retains function and FLP induced recombination between the FRT in the genome and a second FRT containing DNA results in disruption of the first gene (Claim 13). The written description of the invention

describes cells for use in all three of the above strategies; however, the specification fails to disclose how one would carry out all three strategies in animals. Strategies 1 and 3 require introduction of a secondary DNA for homologous recombination at an FRT site. While the specification describes how to introduce said DNA into cells in culture, it does not teach how this is to be done in animals in vivo.

Furthermore, the specification at pages 2-3 creates doubt as it indicates that prior to the instant invention, manipulation of transgenes was impaired due to inability to control site of integration, number of copies, temporal expression and the like. The specification teaches that the instant invention overcomes these impairments. However, the specification does not disclose inserting a FRT site into a gene where the location of the inserted FRT sites is predetermined nor does the specification teach one skilled in the art how to determine such a site. A site for inserting the FRT site must be determined without altering the structure and/or function of the protein encoded by the gene into which the FRT site is to be inserted and the site must be in a region of the genome that contains desired functional activity. Thus, the specification fails to teach how to insert the FRT site specifically into the genome of a mammal as to allow for the utility taught in the specification (pages 2-3).

Claims 1-19 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 1-11 are directed to a transgenic non-human mammal comprising at least one FLP recombination target. Claims 12-19 are directed to a transgenic, non-human mammal comprising at least on FLP recombination target and an additional DNA fragment comprising at least a portion of a gene of interest and at least one FLP site.

The purpose of the claimed transgenic animals are to:1) allow for site-specific insertion of an FRT- transgene construct by homologous recombination with an FRT site within the genome of an animal (claims 1, 3-8, 11, 15, 16; refer to specification page 4, para. 0011), 2) allow for directed activation of a gene by removal of an FRT-flanked disruption using FLP-induced homologous recombination between FRT sites located within said gene (claims 2,9,10,12,17,18; refer to specification page 4, para. 0010), and 3) to insert a gene disruption construct by FLP-induced homologous recombination with a nondisruptive FRT located within a gene of interest (claims 2,9,10,13,14,17,19; (refer to specification page 20, para. 0063). The teachings in the specification demonstrate the use of the FLP/FRT recombination system in mammalian cells, in vitro for purpose 2 (pages 13-19, Examples 1 and 2) and purpose 3 (pages 20-21, Example 3).

The state of the art at the time of filing held that it was unpredictable how to obtain the phenotype of interest in transgenics. The species-specific requirements for transgene design are not clearly understood. Examples in the literature aptly demonstrate that even closely related species carrying the same transgene construct can exhibit widely varying phenotypes. For example, several animal models of human diseases have relied on transgenic rats when the development of mouse models was not feasible. Mullins (1990; Nature, Vol. 344, pages 541-544) produced outbred

Sprague-Dawley x WKY rats with hypertension caused by expression of a mouse *Ren-2* renin transgene. Hammer (1990, Cell, Vol. 63, 1099-1112) describe spontaneous inflammatory disease in inbred Fischer and Lewis rats expressing human class I major histocompatibility allele HLA-B27 and human β_2-microglobulin transgenes. Both investigations were preceded by the failure to develop human disease-like symptoms in transgenic mice (Mullins, 1989, EMBO J., vol. 8, pages 4065-4072; Taurog, 1988, Jour. Immunol., Vol. 141, pages 4020-4023) expressing the same transgenes that successfully caused the desired symptoms in transgenic rats. Thus, the combination of elements (protein, promoter, species of protein, and species of transgenic) required to obtain a desired effect were not within the realm of routine experimentation at the time of filing.

Not only is the difference in transgenic mice and rats unpredictable for reasons stated above, the art at the time of filing was such that a number of significant limitations regarding the production of non-human transgenic animals existed. Wall (1996, Theriogenology, Vol. 45, pages 57-68) disclosed the unpredictability of transgene behavior due to factors such as position effect and unidentified control elements resulting in a lack of transgene expression or variable expression (paragraph bridging pages 61-62). Overbeek (1994, "Factors affecting transgenic animal production," Transgenic animal technology, pages 96-98) taught that within one litter of transgenic mice, considerable variation in the level of transgene expression occurs between founder animals and causes different phenotypes (page 96, last paragraph). Mullins (1996, J. Clin. Invest., Vol. 98, pages S37-S40) teach that non-mouse ES cells capable

of providing germline chimeras were not available (page S38, column 1, first paragraph). Therefore, it was unpredictable at the time of filing what gene of interest, promoter, enhancer, coding, or non-coding sequences present in the transgene construct, site of integration, method used and phenotype obtained were required to make a transgenic non-human mammal of interest.

1) The specification does not enable using any of the 3 strategies described in the specification to make FLP-recombinant transgenics (claims 1-19) because it does not provide the guidance necessary for one of skill in the art to prepare transgenic animals and obtain levels of FLP-recombinase sufficient to induce FLP-mediated recombination in vivo. The specification contemplates introducing FLP recombinase to an animal by administering the enzyme as a protein, by breeding with a transgenic expressing the enzyme, or by transfection with DNA encoding the enzyme (page 12, paragraph 0038). The specification does not teach the levels of FLP recombinase activity needed to reach the nucleus to allow for recombination at FRT sites of an animal. The specification does not address how to deliver the recombinase enzyme to cells of an animal without disrupting recombinase activity by denaturation or degradation. When using transgenesis as a means of delivering FLP recombinase to all cells, as discussed above, the state of the art at the time of filing was that, due to variables such as the promoter, position effect, species and genetic background, the phenotype of a transgenic animal is unpredictable. The state of the art also held that the level of recombination induced by FLP recombinase at FRT sites, in vivo, was dependent upon the level of FLP recombinase expressed (Golic, 1989, Cell, Vol. 59, pp.

499-509, specifically page 507, first full para; Dymecki, 1996, PNAS, Vol. 93, pp. 6191-6196, specifically page 6195, last sentence-page 6196 lines 1-3). Importantly, the specification discloses substantial differences in detectable levels of recombination dependent upon the cell line used (page 17, para. 0055). In vivo, the FLP recombinase gene would be stably integrated in the genome of the animal, as opposed to being introduced as a plasmid, and the amount of FLP recombinase required to cause recombination in vivo and how to achieve this level of expression in vivo is unknown. The specification teaches in vitro transient transfection of cells with plasmid comprising a CMV promoter driven FLP recombinase gene (for example see page 16, para. 0054). The specification does not provide adequate guidance to apply this technique in vivo. Due to the unpredictability of phenotype in transgenics, the unpredictability of the amount of FLP required to obtain recombination in vivo, and the dependence of recombination activity on the level of FLP recombinase obtained, it would require one of skill in the art undue experimentation to determine how to prepare transgenic animals such that sufficient levels of FLP recombinase are expressed to induce detectable of FLP-mediated recombination.

2) The specification does not enable inserting a transgene into a specific locus within the genome. To direct the insertion of a transgene to a specific site within the genome, an FRT site must be provided within the genome that allows for favorable insertion and subsequent expression of the inserted transgene. Random insertion of the FRT site would not work because of position effects (Wall, 1996, Theriogenology, Vol. 45, pages 57-68; Dymecki, 1996, PNAS, Vol. 93, pages 6196-6196) and would not

provide utility over standard transgenic techniques, which also rely on random transgene insertion. When site-specific insertion of a transgene is desired, the FRT recombination site must insert into a known, pre-selected region of the genome that contains and/or allows desired functional activity. While the specification is enabling for site-specific recombination of a transgene with an FRT site located randomly within the genome of cells, it is not enabling for the necessary prior step of determining how and where to place the initial FRT sequences with which the transgene will recombine. Therefore, it would require one skilled in the art, undue experimentation to determine how to insert an FRT into the genome of an animal in any way other than through random insertion.

3) The specification further fails to provide a means for introducing to all cells of a mammal, in vivo, a transgene designed for insertion into an FRT site as the specification described for cells in culture (for example see page 20), and as encompassed by claims 13-19 (purposes 1 and 3). It is not clear how one would provide a transgene to all cells of an animal and have that transgene maintained until a chosen time of FLP recombinase expression. It is possible to readily introduce DNA into cells, in vitro, concomitant with FLP recombinase expression, allowing for temporal control of transgene insertion and subsequent expression or disruption of expression, as described in the specification. Doing so in ES cells would provide a means of generating a transgenic, however, because the FRT site is randomly inserted in the genome (see previous paragraph) of the ES cell, the resulting recombinant is no different than that of a standard technique of generating transgenics. Thus, recombining a transgene into a

randomly inserted FRT site in an ES cell, would not satisfy any of the advantages of this system as taught by the specification such as spatial or temporal control of transgene insertion, activation or inactivation (page 2, paragraph 005) or insertion of transgene into a desired location (page 2, paragraph 004).

4) The specification does not enable the use of a transgenic mammal comprising an FRT site to provide a functional gene by FLP-mediated recombination (purposes 1 and 2). The specification fails to disclose how to insert the β-galactosidase-FRT-disrupted transgene into the genome such that, after excision of the disruption, the active, recombined transgene is expressed to detectable levels. Dymecki (1996) used the strategy described in Figure 1B of the specification (purpose 2) in mice and it did not result in the expected expression of the recombined β-galactosidase transgene. Flp-mediated excision of an inactivating region did occur; however, β-galactosidase activity was not functionally and detectably expressed due to the position effects surrounding the FRT site (Dymecki, page 6196, column 1, lines 43-46), which is random in both the instant invention and in Dymecki. In fact, to avoid this limitation, Awatramani (2001, Nature Genetics, Vol. 29, pages 257-259) targeted an FRT-disrupted PLAP gene to the ROSA26 locus, known to be broadly expressed both spatially and temporally throughout development (Soriano, 1999, Nature Genetics, Vol. 21, pages 70-71), to ensure expression of the activated, recombinant PLAP gene upon FLP-mediated removal of inactivating sequences. It would require one of skill in the art at the time the invention was made, undue experimentation to determine how to insert a transgene such that

levels of expression are such to obtain a desired activity or phenotype upon FLP-mediated transgene activation.

5) The specification does not provide adequate guidance for one of skill in the art to generate transgenic rats, monkeys or hamsters (claims 6-8 and 15-19). As discussed above, the phenotype of a transgenic is unpredictable and closely related species carrying the same transgene construct can exhibit widely varying phenotypes. The specification does not teach how to make and/or use a transgenic rat, hamster, or monkey, comprising an FRT site. The specification does not teach what kind of rat, monkey, or hamsters cells to use to make the transgenic animals. Furthermore, ES cells, which may be required for the claimed invention, are not available in species other than mouse, including monkey, rat and hamster (Mullins, 1996, J. Clin. Invest., Vol. 98, pages S37-S40). Without guidance as to how to generate the claimed rats, monkeys, or hamsters, it would have required undue experimentation for one of skill in the art to make a transgenic rat, monkey, or hamster carrying an FRT for the purposes outlined in the specification (see above) and claim 13.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

1) Claims 1,3-5,15,16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Golic (1989, Cell, vol. 59, pp 499-509) in view of Le Mouellic (1990, PNAS, vol. 87, pages 4712-4716) and Rogers (WO87/03006).

Golic disclosed transforming the germline of Drosophila melanogaster with DNA comprising a marker gene flanked with two FRT sites (Figure 2B). Golic mated the resulting FRT-white-FRT transgenic flies with transgenic flies carrying the FLP recombinase gene under the control of an inducible heat shock promoter to produce flies carrying both transgenes. Expression of the FLP recombinase during larval development in fly larvae carrying both transgenes resulted in excision of the white gene in flies carrying both transgenes (page 501, column 1, lines 2-5). Golic did not teach using such a system to make transgenic mammals.

However, Le Mouellic disclosed transforming the germline of transgenic mice with DNA encoding β-galactosidase (page 4713, "Targeted Replacement Vector" and Figure 1) using embryonic stem cells (page 4715, column 2, lines 24-26), which were introduced into a developing embryo (page 4715, column 2, lines 24-26) and resulted in transgenic mice carrying DNA encoding β-galactosidase .

It would have been obvious to one of skill in the art at the time the invention was made, to make a transgenic having a transgene comprising a marker gene flanked by two FRT sites and a transgene comprising DNA encoding FLP recombinase, as taught by Golic, to make a transgenic mouse using ES cells as taught by Le Mouellic. One of ordinary skill in the art at the time the invention was made would have been motivated to introduce the FLP/FRT recombination system into a mouse to allow for "the regulated

expression of heterologous genes" in mice as suggested by Rogers (page 2C, lines 3-6). One of ordinary skill in the art at the time the invention was made would be motivated to generate a mouse using the FLP/FRT recombination system to allow for greater spatial an temporal control (Rogers, Abstract and page 14, lines 12-17) in generating mice that would allow for study of gene expression or function at a time in development after traditional knockout or introduction of gene expression of a particular gene would have been fatal. One of ordinary skill in the art at the time the invention was made would be motivated to generate a mouse capable of excising a gene disruption leading to overexpression at a time after birth because these alterations are often lethal when introduced during embryonic development. One of ordinary skill in the art at the time the invention was made would be further motivated to generate a mouse using the FLP/FRT recombination system to obtain an in vivo mouse model that is more closely related to human disease. Whereas traditional transgenic knock-in or knockout techniques can affect and alter embryonic development prior to the onset of disease in adults and do not always mimic the genetic alterations correlated with disease states either spatially or temporally, the claimed invention would allow for specific genetic alterations that can be spatially and temporally induced in adult mice that, as a result, more closely resemble those that occur in natural disease states.

Thus, Applicants' claimed invention as a whole is *prima facie* obvious in the absence of evidence to the contrary.

2) Claims 1-5, 9-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Golic (1989, Cell, vol. 59, pp 499-509) in view of Le Mouellic (1990, PNAS, vol. 87, pages 4712-4716) and Rogers (WO87/03006).

The combined teachings of Golic and Le Mouellic taught a transgenic mouse with a transgene having the β-galactosidase gene flanked by two FRT sites and a transgene encoding FLP recombinase. Motivation to combine Golic and Le Mouellic was provided by Rogers and for other reasons described above. The combined teachings of Golic and Le Mouellic did not teach placing the FRT sites inside a gene of interest.

However, Rogers disclosed FLP recombination target sequences within a gene to allow for regulated expression (page 17, lines 8-23 and Figure 5A) of the gene.

It would have been obvious to one of skill in the art at the time the invention was made, to make a transgenic mouse having a transgene comprising a marker gene and FRT sites in addition to a transgene comprising DNA encoding FLP recombinase, as taught by Golic and Le Mouellic, wherein the FRT sites are within the β-galactosidase gene as taught by Rogers. One of ordinary skill in the art at the time the invention was made would have been motivated to combine the teachings of Golic and Le Mouellic with the teachings of Rogers to introduce the FRT sites within the transgene to allow for "the regulated expression of heterologous genes" (Rogers, page 2C, lines 3-6). One of ordinary skill in the art at the time the invention was made would be motivated to generate a mouse capable of excising a gene disruption leading to overexpression at a time after birth because these alterations are often lethal when introduced during embryonic development. One of ordinary skill in the art at the time the invention was

made would be further motivated to generate a mouse using the FLP/FRT recombination system to obtain an in vivo mouse model that is more closely related to human disease. Whereas traditional transgenic knock-in or knockout techniques can affect and alter embryonic development prior to the onset of disease in adults and do not always mimic the genetic alterations correlated with disease states either spatially or temporally, the claimed invention would allow for specific genetic alterations that can be spatially and temporally induced in adult mice that, as a result, more closely resemble those that occur in natural disease states.

Thus, Applicants' claimed invention as a whole is *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Valarie Bertoglio whose telephone number is 703-305-5469. The examiner can normally be reached on 7:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Reynolds can be reached on 703-305-4051. The fax phone numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications.

Valarie Bertoglio
Patent Examiner

MICHAEL C. WILSON
PATENT EXAMINER